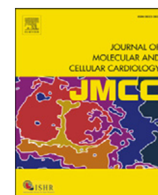
Journal of Molecular and Cellular Cardiology 66 (2014) 133–140

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc



Original article

The time-of-day variation in vascular smooth muscle contractility depends on a nitric oxide signalling pathway

M. Denniff, Helen E. Turrell, Andrew Vanezis, Glenn C. Rodrigo *

Department of Cardiovascular Sciences, University of Leicester, Glenfield General Hospital, Leicester, UK

ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form 7 November 2013
Accepted 11 November 2013
Available online 18 November 2013

Keywords:
Nitric oxide synthase
Mesenteric arteries
Circadian clock
Alpha1-adrenergic receptor
Vasoconstriction

ABSTRACT

The dip in blood pressure during the resting-period is paradoxically associated with an increase in total peripheral resistance and occurs at a time when the vascular response to vasoconstrictor compounds is heightened, and to vasodilators reduced. However, the cellular mechanisms responsible for this time-of-day variation are not well defined. We have investigated the role of nitric oxide synthase (NOS) signalling in the control of contraction in mesenteric resistance arteries using wire myography, combined with quantitative PCR analysis of gene transcription and western blot analysis of protein. Small rings of mesenteric arteries, isolated from rats at two opposing time-points corresponding to the animal's active and resting-period, were mounted in a wire myograph. Vessels exhibited a time-of-day variation in their contractile-response to phenylephrine, with a reduced maximal contraction during the active- versus the resting-period (11.8 ± 0.8 versus 18.6 ± 1.2 mN $P < 0.001$). Vessels preconstricted with phenylephrine were also more responsive to vasodilation with acetylcholine during the active-period, with an EC_{50} of 58.6 ± 11 versus 232 ± 31 nM in resting-period vessels ($P < 0.0001$). These differences were abolished in the presence of L-NAME. Quantitative RT-PCR reveals a functioning peripheral circadian clock in mesenteric arteries and a 3.3-fold increase in endothelial NO synthase mRNA levels in active- versus resting-period vessels ($P < 0.001$), which translated to a 1.7-fold increase in total eNOS protein ($P < 0.05$). The time-of-day variation in the response of mesenteric resistance vessels to phenylephrine and acetylcholine is dependent on NOS signalling.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The presence of circadian rhythms in many haemodynamic parameters has been shown in both animals and humans. This is characterised by a nocturnal “dip” in blood pressure (BP) associated with a fall in cardiac output [1,2] but a paradoxical increase in total peripheral resistance (TPR) in primates [3], humans [4] and in the nocturnal rat during the day [5]. This dip in BP is suggested to protect the individual against cases of sudden cardiac death, stroke and MI, and the absence of the pronounced fall in BP in so-called “non-dippers” has been speculated to contribute to an increase in these events [6,7].

The fall in cardiac output has been linked to the decrease in heart rate (HR) and stroke volume (SV) driven by the diurnal fall in sympathetic activity and not a simple reduction in physical activity, as it is still present in human volunteers confined to bed for 24 h [8]. In rats this pattern of circadian variation in BP and SV is still seen in animals under constant light conditions [6] and is lost following lesion of the suprachiasmatic nucleus (SCN) [9], showing the importance of the central circadian clock. Furthermore, this fall in BP is not directly related to a fall in HR as it is still seen in paced patients [10] and could therefore reflect changes in SV.

The increase in TPR is suggested to limit the fall in BP against the background of a substantial fall in cardiac output [4] and has been attributed to auto-regulation and an absence of muscular activity at night in humans [4]. However, changes in autonomic function that are known to occur over 24 h and the response of the vasculature to vasoactive substances may also have a role to play. Peripheral resistance reflects vascular tone set by the contractile status of vascular smooth muscle (VSM) and this is in turn controlled by an interaction between the intrinsic and extrinsic constrictor mechanisms and their modulation by endothelial secretions, vasoactive metabolites and autacoids. Early studies revealed a strong circadian rhythm in the sensitivity of the rat aorta to in-vitro application of vasoconstrictor and endothelial-dependent vasodilator agents [11,12]. It is possible that the circadian variation in TPR results from a time-of-day variation in the responsiveness of the VSM to neurotransmitter substances (noradrenaline, adrenaline) and/or its subsequent modulation by endothelial function (acetylcholine, shear stress), rather than a simple variation in the activity of the autonomic nervous system.

A time-of-day variation in endothelial-dependent relaxation of VSM is suggested from experiments showing that the acetylcholine-induced increase in forearm blood flow exhibits time-of-day variation, with a peak response at 0800 and trough at 2000 h, and the depression of forearm blood flow in response to nitric oxide synthase (NOS)-inhibition with L-NMMA was also greater at 0800 than 2000, suggesting a diurnal variation in endothelial NOS-signalling [13]. However, whether this

* Corresponding author. Tel.: +44 1162563028; fax: +44 1162875792.
E-mail address: gcr4@le.ac.uk (G.C. Rodrigo).

0022-2828/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.yjmcc.2013.11.009

time-of-day variation in forearm blood flow reflects changes in contraction of the VSM is not known. Acetylcholine binds to receptors on the endothelial cells resulting in an increase in $[Ca^{2+}]_i$ that activates NOS and the production of NO, which diffuses to the VSM causing relaxation through direct actions on BK_{Ca} channels [14] and indirectly through cGMP [15]. The increase in cGMP levels also causes vasodilation through activation of PKG, leading to inhibition of L-type Ca^{2+}-channels and IP_3 receptors and an increase in myosin light chain phosphatase [16]. It is therefore possible that this increase in response to acetylcholine reflects an increase in endothelial NOS–cGMP signalling during the active-period, which is supported by the finding that plasma and tissue levels of cGMP exhibit circadian cycling [17,18].

In this study, we have set out to determine whether the 24-hour variation in vascular contractility in response to α1-ADR activation and subsequent modulation by NOS-signalling exist at a cellular level. We have used wire myography to measure contraction in mesenteric resistance vessels isolated from Wistar rats in response to phenylephrine and relaxation by acetylcholine, at two opposing time points during the animal's inactive- and active-periods. The role on NOS-signalling in the time-of-day variation in the response was probed using the non-specific inhibitor L-NAME.

2. Methods

Male Wistar rats were housed in environmentally controlled rooms (12 hour light/dark cycle) and randomly assigned to one of two separate rooms with opposing light cycles (normal-cycle lights on at 6.00 and reverse-cycle lights on at 18.00). Animals were stunned by a blow to the head followed by cervical dislocation at 9:00 AM, equivalent to a *Zeitgeber time* of ZT3 (resting-period myocytes) for animals in the normal-light cycle or ZT15 (active-period myocytes) in the reverse-light cycle room. The investigation complied with the university's animal care and welfare guidelines, which conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23 revised 1996).

2.1. Myography

For recordings of contractile force, 2–4 mm ring segments of the superior mesenteric artery 1st order branches were mounted in a wire-myograph at 37 °C (model 610 M; Danish Myo Technology). Arteries were stretched radially to an optimal equilibrated passive tension of 5 mN [19]. Equilibrated and static tension was observed for a period of at least 10 min before activation with KCl (60 mM). Vessels were discarded if a contraction of >4 mN was not reached.

2.2. Experimental protocol

2.2.1. Protocol-1

Concentration–response curves of contraction to phenylephrine were obtained by the addition of phenylephrine in stepwise increasing concentration, (range 1 nM to 0.3 mM). Force readings were made at steady-state after each increase in concentration. The organ bath was washed 4 times with physiological saline solution (PSS) after $[Phe]^{max}$ was reached and vessels allowed to equilibrate for 20 min.

2.2.2. Protocol-2

To determine the concentration-dependent relaxation by acetylcholine, a Concentration–response curve was obtained using protocol-1. Vessels were then pre-contracted with a concentration of phenylephrine to cause 70% of maximal response and left whilst contraction settled. Acetylcholine (Ach) was added in stepwise increasing concentration, (1 nM to 0.3 mM). Force readings were made at steady-state after each increase in concentration.

2.3. Determination of mRNA expression

Entire superior mesenteric artery cascades were dissected and snap frozen in liquid nitrogen. mRNA expression was analysed and quantified using fluorescent probe-based Taqman gene expression assays. (*For detailed methods see supplemental methods and data section and Collins and Rodrigo* [20].)

2.4. Western blotting

Entire superior mesenteric artery cascades were homogenised and lysed in RIPA buffer, followed by electrophoresis on 10% SDS-PAGE gel. Proteins separated on the gel were electrotransferred to nitrocellulose membrane (Amersham Biosciences,) at 4 °C for 1 h at a constant voltage of 100 V. The membranes were blocked by rocking with 5% nonfat dry milk in Tris-buffered saline and Tween 20 (0.01%) (TBS-T). Primary antibodies were incubated overnight at 4 °C. Secondary antibodies were goat anti-mouse IgG and goat anti-rabbit IgG (1:2000, Sigma-Aldrich) for 1 h at room temperature. Membranes were incubated with ECL and exposed to autoradiography film. Bands were quantified using a GeneGenius Bioimaging System with GeneSnap and GeneTools software (Syngene).

2.5. Drugs and solutions

The physiological saline solution was of the following composition (mM): NaCl 137, KCl 5.4, Na_2HPO_4 0.44, NaH_2PO_4 0.42, Glucose 4, Mannitol 6, [N-(2-hydroxyethyl) piperazine-N′-[Z-ethane sulphonic acid] (HEPES) 10, $MgCl_2$ 1, adjusted to pH 7.40 with NaOH. KCl was added to increase the concentration to 60 mM and NaCl reduced in an equimolar amount. Mouse anti-eNOS was obtained from BD Biosciences. Rabbit anti-β3-AR antibody was obtained from Santa Cruz Biotechnology. Mouse anti-α-tubulin was obtained from Sigma-Aldrich. Stock solution of Acetylcholine-HCl (Ach), phenylephrine (Phe) and L-Nitro-Arginine Methyl Ester (L-NAME) and BRL-37344 were prepared and added to the PSS to the required concentration by serial dilution (Sigma Aldrich).

2.6. Statistical analysis

Maximal and percentage responses are expressed as mean ± S.E.M. The number of animals in each group is represented by n = animals/vessels. EC_{50} was obtained from curve-fitting non-linear fit; log (agonist) versus response-variable slope using the graphical package GraphPad Prism. Data were compared, as appropriate, using a 2-way ANOVA and Bonferroni *post-hoc* test or Student's unpaired *t*-test.

3. Results

3.1. Diurnal variation in the response of mesenteric arteries to phenylephrine: role of NO-signalling

The contractile response of rat and mouse aortic strips to phenylephrine and high K^+ exhibits a time-of-day variation, with a reduced response during the active-period [21]. However, the presence of a diurnal variation in contraction of resistance vessels and hence peripheral resistance and blood pressure has not been shown. We therefore set out to determine the impact of the time-of-day, on the contractile response of small rat mesenteric arteries to phenylephrine, a powerful vasoconstrictor which binds to α1-ADR on the surface of VSM cells activating contraction, and which is then modulated by endothelium derived vasodilator substances such as NO, EDHF, and prostanoids.

Concentration–response curves of contraction strength to increasing concentrations of phenylephrine were constructed from vessels isolated during the animals' resting- and active-periods, and show a time-of-day variation in the amplitude of contraction in response to a concentration of phenylephrine >3 μM, with a maximal amplitude of contraction

Download English Version:

https://daneshyari.com/en/article/2190559

Download Persian Version:

https://daneshyari.com/article/2190559

Daneshyari.com